THE DISTRIBUTION OF BACTERIA IN BOTTLED MILK AND CERTAIN CONTROLLING FACTORS.*

JOHN C. TORREY AND ALFRED H. RAHE.

(From the Department of Experimental Pathology, Loomis Laboratory, Cornell University Medical College, New York.)

IT has been known for some time that the cream of cow's milk is always much richer in bacteria than the leaner portions, but as Anderson[1] has remarked and as his review of the scanty literature shows, the fact of this unequal distribution has attracted very little attention. The results of recent investigations of Hess[2] and later of Anderson, however, suggest that this excessive bacterial contamination of the top milk may be of some import in the etiology of infantile summer intestinal disturbances, as it is a common practice to use this portion of bottled milk in the preparation of modified milk for bottle-fed infants. Anderson has found in an examination of a large number of samples of Washington milk that both gravity and centrifuged cream contain from 10 to 500 times as many bacteria as the mixt milk.

With the purpose of mitigating in a measure the possible danger to infants of ingesting such excessive numbers of bacteria, Hess[3] has advised that the first two ounces from the bottle of milk be removed and set aside for table use, and that the following 7, 8, or 12, ounces (giving respectively 12 per cent, 10 per cent, and 7 per cent cream) alone be used in infant feeding. He believes that this procedure is justified through his finding that the top layers of cream have a higher bacterial content than the subjacent portion.

In this paper are embodied the results of the examination of a large number of quart bottles of milk (over 90) which were in some degree representative of the average bottled milk supply of New York City during the past summer, ranging in quality from that supplied by

* Received for publication January 3, 1910.

[1] *Jour. Infect. Dis.*, 1909, 6, p. 392.

[2] *Archives of Pediatrics*, 1908, p. 31.

[3] *Jour. Amer. Med. Assoc.*, 1909, 53, p. 523.

shops on the "East Side" to the highest quality of certified milk. This work was undertaken at the suggestion of Dr. Joseph E. Winters and with the aim of determining:

a) In greater detail the relative distribution of bacteria in bottled milk especially as regards the cream layers;

b) To what degree the bacterial count is decreased by following the procedure of Hess;

c) What are the more important factors which influence the disposition of the bacteria in the bottle.

Methods.—The bottles of milk were obtained either from many sources or at times from a single dairy, as best served the purpose of the experiments. After delivery at the laboratory these bottles had stood for two hours or more in the ice-box before sampling so that there was always a distinct line of demarkation between the cream and the skim milk.

For plating, dilutions of the milk were made in sterile normal salt solution in multiples of ten. At each step the test tubes were shaken vigorously and stirred with the pipette to break up the clumps of bacteria which are so frequently present in milk and which, left intact, give a false impression as to the degree of bacterial contamination. In each instance 1 c.c. of the dilution was plated. The media, with the exception of the litmus lactose agar, was titrated to 1.5 acid to phenolpthalein as recommended by the Committee on Bacterial Milk Analysis. In the majority of the experiments meat infusion peptone broth served as the basis for the agar and the gelatin, altho in the earlier work the nutrient agar was prepared with Liebig's extract of beef. A comparative series disclosed a slightly lower average count with the "extract" agar, and hence it was discarded. Agar of the percentage of 1.2 and gelatin of 10 were used.

The agar plates were incubated for 24 hours at 37° C., and the gelatin, for three days at room temperature. With certain of the agar plates a supplementary incubation at room or 27° C. temperature was given. As has been shown by Wilcox[1] and others, a higher average count is obtained by the longer incubation at the lower temperature, but as the increased growth was quite evenly distributed through the cream and the skim milk, for the purpose of these experiments the shorter incubation at 37° C. served fully as well.

Samples from different parts of the milk bottle were obtained in several different ways. In the first series the cruder procedures were followed, which give an insight into the numbers of bacteria in milk as commonly fed to infants. In the second series smaller samples were obtained with less disturbance of the milk.

1. Samples obtained with a 50 c.c. pipette.—This mode of sampling was used in a series of 10 bottles of milk of various degrees of contamination. The first four samples were removed consecutively (the first three consisting entirely of cream and the fourth of cream

[1] *Studies from the Research Laboratory, Department of Health, City of New York*, 1907, 3, p. 186.

with a small admixture of skim milk). The fifth sample consisted of the middle skim milk and the sixth of the sediment portion.

2. Sampling with an ounce milk dipper.—In a series of 12 bottles of ordinary milk from various dairies in the city the fluid was dipped out ounce by ounce with an ounce milk dipper, using no greater caution against mixing and stirring the fluid than would be taken in the everyday preparation of an infant's diet. From four to six consecutive ounces were dipped from the top of the bottle, one from the middle and one from the sediment portion. In certain instances all of the dipped samples were added to the remaining milk and the whole mixt and plated to determine the bacterial count for the "whole milk."

TABLE 1.
DISTRIBUTION OF BACTERIA IN BOTTLED MILK.

Samples	Samples Obtained with a 50 c.c. Pipette			Samples Obtained with a 1-Ounce Milk Dipper		
	Average of 10 Experiments	Average of 5 Low-Count Bottles	Average of 5 High-Count Bottles	Average of 12 Experiments	Average of 5 Low-Count Bottles	Average of 7 High-Count Bottles
Top	827,000	147,500	1,523,700	4,745,000	509,200	7,771,000
Second	964,500	101,000	1,828,000	5,397,000	552,500	8,857,000
Third	596,700	17,400	1,177,600	2,918,000	413,800	4,707,000
Fourth	270,440*	19,750	468,400	2,493,000	302,900	4,043,000
Fifth	....	....	....	2,470,000†	....	....
Sixth	....	....	....	1,960,000†	....	....
Middle skim milk	17,850‡	17,500	....	428,000	112,000	654,000
Sediment	153,850	21,400	286,800	521,000	131,675	819,000

* Nine experiments only.

† Four experiments only.

‡ Six experiments only.

3. Samples poured from bottle.

TABLE 2.
SAMPLES OBTAINED BY POURING OR DIPPING VARIOUS AMOUNTS OF THE MILK.

Samples	First 2 Ounces Poured Off and Following Samples Dipped with 1-Ounce Dipper		Sampled by Pouring. Each Sample 1 Ounce	Sampled by Pouring. Each Sample 2 Ounces
	Average 5 Experiments	Exceptional Count, not Averaged	Average 3 Experiments	Average 5 Experiments
1. First 2 ounces	1,025,000	730,000	284,000 137,000	460,000
2. Third ounce	1,434,000	9,100,000	64,000	116,000
3. Fourth ounce	1,288,000	2,300,000	120,000	
4. Fifth ounce	1,204,000	500,000	....	90,000
5. Sixth ounce	454,000	320,000	....	
6. Skim milk	67,000	200,000	17,000	36,000

The following chart illustrates graphically the very marked difference in the degree of the bacterial contamination in the cream layers as compared with the skim milk. In this chart the count for the middle skim milk is used as the unit of measure and the ordinates express the ratio of the bacterial content of other portions of the

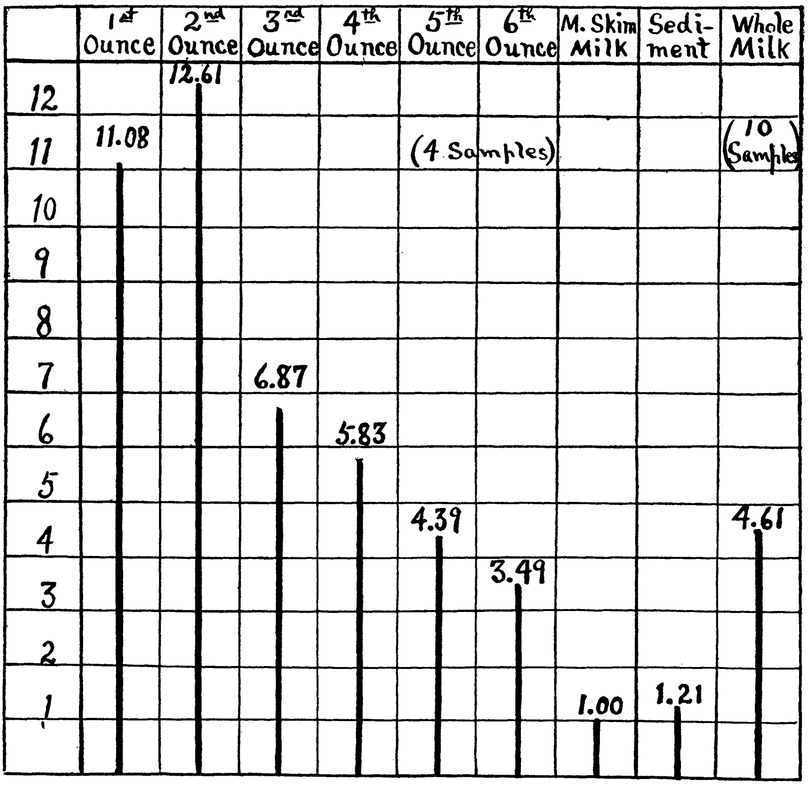

CHART 1.—Showing the difference in the degree of contamination of various cream layers and the skim milk.

bottle and of the mixt milk to this unit.[1] If more grossly contaminated milk had been used as the basis of this chart, without doubt the differences in the length of the lines would have been much greater, but it seems probable that it expresses fairly accurately the disposition of bacteria in the average bottle of fresh milk with a count of about 1,000,000 bacteria per c.c.

[1] Unless otherwise stated the ratios were determined from the average count of 12 samples of medium grade milk.

The general average of these pouring and dipping experiments disclosed a preponderance of bacteria in the second ounce of cream over the first. On the contrary the counts given by Hess show this relation regularly reversed in that the first ounce of cream embodied on the average over 100 per cent more bacteria than the second. Without doubt this discrepancy is due to the fact that according to his tables the samples of milk examined by him contained in general fewer bacteria than did ours. We have found that when the upper cream averaged under two million bacteria per c.c. the conditions outlined by Hess obtained, but when the count was much over that mark the second ounce often revealed a 100 per cent higher bacterial content than the first.

These first two ounces, however, as Hess has maintained, frequently contain many more bacteria than the lower layers of the cream, for with the third ounce there is generally a marked diminution in the count which continues as we approach the skim milk. At times, nevertheless, one encounters a striking exception to this generalization, a good example of which is given in the second column of Table 3. In this bottle the third ounce averaged over 12 times the count of the first two ounces.

4. Samples obtained with a 1 c.c. pipette.—In dipping samples from the bottle the fluid is unavoidably more or less disturbed with a consequent redistribution of bacteria. Samplings with a 1 c.c. pipette, carefully performed, give a truer picture of the actual bacterial contamination of the various strata. The results, however, are of more theoretical than practical interest.

The various depths at which samples were desired were indicated by marks on a sterile pipette, measured from the tip. After rinsing with sterile salt solution, and with the thumb tightly pressed over the mouth end, the pipette was sunk vertically into the fluid until the surface reached the measured mark. The milk was then drawn up in a little excess of 1 c.c., the pipette removed and its exterior thoroughly rinsed off. Discarding the excess, the 1 c.c. sample was diluted and plated. Experience indicated that this is a satisfactory and reliable method for the purpose of the experiment.

The results with this method of sampling are in accordance with those obtained by dipping, in that they reveal an excess of bacteria

in the upper third of the cream, as compared with the lower portions. The samples from the surface layer, constituting what might be

TABLE 3.

DISTRIBUTION OF BACTERIA IN BOTTLED MILK. SAMPLES OBTAINED WITH A 1 C.C. PIPETTE.

Average Bottled Milk			Certified Milk			Exceptionally "High Counts"	
Samples	Average of 7 Experiments	Ratio to Sample 6 Top Skim	Samples	Average of 13 Experiments	Ratio to Sample 5 Middle Skim	Average of 2 Experiments	Ratio to Sample 5 Middle Skim
Cream:							
1. Surface	510,000	17.00	1. Top cream...	22,700	51.49	11,960,000	2.40
2. ½ inch......	406,000	13.53	2. Middle cream	15,370	34.93	6,860,000	1.38
3. 1 inch......	288,000	9.60	3. Bottom cream	10,390	23.61	3,900,000	.78
4. 2 inches....	310,000	10.33	4. Top skim....	1,030	2.34	3,900,000	.78
5. 3 inches....	164,000	5.46	5. Middle skim .	440	1.00	4,970,000	1.00
Top skim milk:							
6. 4 inches....	30,000	1.00	6. Sediment	320	.72	2,400,000	.48
Sediment:							
7. 7 inches....	18,000	60					

called the scum of the cream, show the maximum counts, and as the samples approach the lean milk here is a gradual but frequently irregular drop in the degree of contamination.

PRACTICABILITY OF DISCARDING THE FIRST TWO OUNCES OF CREAM.

These various methods of sampling have shown that, as Hess contends, there is not a homogeneous distribution of bacteria in the cream, but the greater numbers are, on the average, in the top layers. In Table 4 is indicated the degree to which milk, for infant feeding, is improved bacteriologically by discarding the upper two ounces. These figures are not absolutely correct but are sufficiently so for the purpose in view. The error is always toward the side of indicating greater reduction than actually occurs. In each instance the cream is calculated as six ounces.

By discarding the first two ounces of cream and utilizing the next eight ounces (10 per cent cream) instead of the first eight ounces, we find in a series of 35 quart bottles of various grades of milk, sampled by four different practical and necessarily crude methods, that there occurs a decrease of 29 to 51 per cent in the bacterial content. It seems questionable if this degree of diminution is sufficient

to be of service in the purification of milk for infant feeding. The cream of New York City bottled milk contains during the hot summer weather a probable average of over 5,000,000 bacteria per c.c. A reduction of between one-third and one-half in this contamination would not materially improve the chances of the infant against the intestinal disturbances associated with an impure milk, nor would it approximate in more than negligible degree the desirable limit of bacterial contamination in milk for infant feeding which Coit has

TABLE 4.

PERCENTAGE OF DECREASE OBTAINED BY DISCARDING THE TWO TOP OUNCES OF THE CREAM.

Method of Sampling	Average Bacteria per c.c. First 8 Ounces	Average Bacteria per c.c. in the 8 Ounces Below the First 2	Percentage of Decrease According to Hess's Method
1. Pipetting—50 c.c.	(First 250 c.c.) 535,000	(First 50 c.c. discarded) 373,000	31 per cent
2. Pouring—1 ounce	94,950	54,500	46 " "
3. Pouring—2 ounces	92,950	69,500	25 " "
4. Dipping—1 ounce; 1 to 4 consecutive	High 4,920,000	2,514,000	49 " "
	Low 363,000	235,000	36 " "
5. Dipping—1 ounce; 1 to 6 consecutive	3,539,000	1,743,000	51 " "
6. Pouring first 2 ounces and dipping the others	820,000	581,000	29 " "

placed at 10,000 per c.c. If milk had become infected with B. typhosus at the farm or dairy the greater number of the bacilli would without doubt be found at the start in the upper two ounces of the cream, but by the time the milk had reached the consumer sufficient multiplication would probably have taken place to render the lower portion of the cream quite as dangerous as the upper. It is possible, however, that the danger from bovine tuberculosis might be mitigated in some degree by following the recommendation of Hess. The milk, in this instance, receives the primary infection at the time it is drawn from the cow, and as, in all probability, there would be no subsequent multiplication of the tubercle bacilli in the milk a considerable excess of them would be carried up and embodied in the upper layers of the cream. Hence the chances of infection might be somewhat greater when this top portion is utilized, than with the lower cream or the mixt milk.

FACTORS INFLUENCING THE DISTRIBUTION OF BACTERIA IN BOTTLED MILK.

MECHANICAL ACTION OF RISING CREAM.

The "rafting" activity of rising cream is, of course, the prime factor in effecting the higher degree of contamination of top milk. A number of experiments were carried out to determine the degree of effectiveness of the rising fat globules in entangling and filtering out bacteria from skim milk and to what extent the lean milk is purified in the process.

In one set of experiments a bottle of milk, in which the cream had completely separated, was sampled by the 1 c.c. pipette method at six depths (top, middle, and bottom cream and the same layers for skim milk). The proper dilutions were plated in agar, in gelatin, and in litmus lactose agar. Next the entire content of the bottle was poured into a flask and thoroughly mixt. A sample of this whole milk was plated in each medium. It was then poured back into the bottle and placed at 5° C. Platings were made at the various intervals designated in Table 5. The samples were taken at the same levels as those obtained before the shaking, these levels having been indicated by marks on the pipette. In this tabulation the ratio of change in the count (increase or decrease) from that of the mixt milk control is given for each period and media instead of the actual number of bacteria. In this way not only is space economized but a clearer impression is conveyed of the movements of the bacteria in the bottle through the agency of the cream.

Table 5 indicates that, five hours after the shaking, the bacteria have resumed practically the same distribution in the milk bottle which they held at the beginning of the experiment. A noteworthy difference, however, is present in the fact that the top cream has become proportionally richer in bacteria. It is likely that this local increase is due to the fact that the milk contains now more bacteria than at the primary rising of the cream, and hence a greater proportion would be rafted upward and incorporated in the first cream layers. The 24-hour plating shows that there has occurred a further bacterial contamination of the cream in correlation with a continued purification of the skim milk. A certain amount of this increase in the cream at 24 hours may be the result of multiplication, especially of

the lactic acid bacteria. All tendency toward sedimentation has been more than neutralized by the lifting properties of the cream, as is clear from the fact that the bottom of the bottle after 24 hours contains a smaller average number of bacteria than before it was shaken.

TABLE 5.

REDISPOSITION OF BACTERIA IN BOTTLE AFTER SHAKING MILK.

Whole (shaken) milk { Agar, 143,000 bacteria per c.c.; Gelatin, 130,000 bacteria per c.c.; Lit. lact. agar, 39,000 bacteria per c.c.

SAMPLES (WITH 1 C.C. PIPETTE)	BEFORE SHAKING RATIOS TO COUNTS WITH WHOLE MILK			½ HR. AFTER SHAKING	1 HR. AFTER SHAKING			2 HRS.	5 HRS.			24 HRS.		
	Agar	Gelatin	Litmus Lactose Agar	Agar	Agar	Gelatin	Litmus Lactose Agar	Agar	Agar	Gelatin	Litmus Lactose Agar	Agar	Gelatin	Litmus Lactose Agar
1. Top Cream	2.54	4.33	3.66	.38	.73	1.30	1.59	3.63	3.63	3.77	6.15	4.54	7.92	8.46
2. Middle cream..	2.93	3.92	4.66	.36	.52	.84	1.20	1.53	2.44	2.92	3.66	2.51	6.30	5.89
3. Bottom cream..	2.44	2.61	3.33	.28	.49	1.00	.97	.55	2.51	2.77	3.00	2.93	5.46	6.66
4. Top skim milk....	.25	.28	.28	.55	.54	.89	1.07	.72	.33	.23	.30	.12	.19	.21
5. Middle skim milk	.04	.11	.15	.53	.58	.87	1.05	.49	.35	.20	.33	.08	.20	.18
6. Bottom skim milk	.10	.13	.11	.51	.51	.63	1.02	.28	.12	.12	.20	.03	.05	.05

In this and other similar experiments there occurred a reduction of one-half to one-third in the average count one-half hour after shaking. It seems probable that this apparent decrease may be largely the result of clumping of the bacteria rather than an actual destruction. After one hour there was no distinct cream layer and only a slight increase of bacteria at the surface. It is, in fact, only when the cream layer begins to form that we find an excess of bacteria on the surface. That they collect on the top exactly *pari passu* with the fat globules was especially noticeable in two hours when the top layer of cream contained per c.c. several times the number in the skim milk and actually more than were present in this layer before the shaking.

COMPARISON OF THE CHANGE IN POSITION OF BACTERIA IN WHOLE MILK CONTAINING VARIOUS PROPORTIONS OF CREAM.

About one-fifth of the fluid in the average bottle of dairy milk is cream. The object of the following experiment was to determine

whether cream in less or greater ratio than this filtered out a proportional ratio of bacteria, and further to ascertain whether milk bacteria tend to collect at the surface of skim milk devoid of cream.

A bottle of fresh dairy milk was placed in the ice-box for three or four hours. The cream was then pipetted off and heated in a water bath at 65° C. for one hour. The remaining unheated skim milk was next poured into a large globular separating funnel and placed at 5° C. over night as was also the partially sterilized cream. Next morning the skim milk was drawn off very carefully and collected practically free from cream. After shaking it thoroughly a sample was plated in plain agar, gelatin, and litmus lactose agar. The partially sterilized cream was subjected to the same procedure. The mixtures of these two fluids described in the heading of Tables 7 were then made in 100 c.c. amounts and each placed in a sterile glass-stoppered bottle of that capacity. In addition, two bottles were filled with 100 c.c. of the contaminated skim milk alone, to serve as controls. The several bottles were shaken thoroughly and placed at 5° C. Platings were made with three media at the time intervals indicated in the following table. From each bottle samples of 1 c.c. were taken from the top, the middle, and the bottom. The ratios of change in this table were calculated by dividing the number of bacteria in each sample at the various periods by the average number present in each bottle at the beginning of the experiment, determined theoretically on the basis of the proportions of cream and skim milk in the several mixtures.

Table 6 indicates that the extent of the primary rise of bacteria in whole milk is correlated with the measure of its cream content With "mixture 4" a much higher proportion of bacteria were present in the two-hour cream than with mixtures 1 or 2. This is as one might anticipate, as in the former instance the fat globules, being much more numerous, are closer together and hence would entangle and raft upward a larger proportion of bacteria. At the end of two hours, when from one-half to three-fourths of the total cream has risen, these differences are especially marked. In not all of the experiments of this character was this point brought out as clearly. The degree of contamination of the skim milk is a factor of importance; when this is high the differences between the skim milk and the cream would be much less. At 5 and 24 hours the average of the ratios calculated for each of the three media is placed in the table.

In these experiments no special type of bacteria, such as those growing best at room temperature, the gelatin liquefiers, and the lactic-acid formers, exhibited any special predilection for the cream layer. In fact, after 24 hours, there was a higher average percentage of the gelatin liquefiers at the middle and bottom of the bottle than at the top. Taken all in all, however, their position as well as that

of other types of milk bacteria seems to be quite fortuitous. It may be concluded then that, of the number of pathogenic bacteria which gain access to the milk before it is finally bottled, approximately the same proportion would be carried up and incorporated in the cream as is indicated in the table for the harmless saprophytes.

TABLE 6.

INFLUENCE OF VARIOUS AMOUNTS OF CREAM ON THE DISTRIBUTION OF BACTERIA.

Mixture 1. Partially Sterilized Cream 10 c.c., Skim Milk (Contaminated) 90 c.c. = 1–10.
" 2. " " " 15 " " " " 85 " = 1–6.6.
" 3. " " " 20 " " " " 80 " = 1–5.
" 4. " " " 25 " " " " 75 " = 1–4.
Control 1. No Cream, Skim Milk 100 c.c.
" 2. " " " " 100 "

	2 Hours	5 Hours	24 Hours		
	Ratios to Preliminary Counts. Agar	Ditto. Average of Ratios with 3 Media	Ditto. Average of Ratios with 3 Media	Percentage of Liquefiers	Ratio of Increase of Liquefiers
Mixture 1:					
Top	2.81	4.50	6.41	21.1	6.33
Middle	1.97	.60	3.77	28.2	1.83
Bottom	1.52	1.14	1.56	49.1	4.66
Mixture 2:					
Top	6.85	7.00	23.23	19.7	12.50
Middle	3.03	.59	.96	37.5	2.00
Bottom	2.37	.47	5.74	18.0	3.00
Mixture 3:					
Top	10.43	10.14	12.31	18.9	8.33
Middle	1.27	.50	.85	33.3	1.33
Bottom	1.27	.69	1.00	43.3	2.18
Mixture 4:					
Top	17.49	10.41	28.12	26.6	20.00
Middle	1.93	.77	.99	41.1	1.16
Bottom	2.09	1.18	1.50	13.9	.83
Control 1:					
Top	1.95	1.47	3.13	20.	3.66
Middle	2.99	.89	2.21	31.8	5.83
Bottom	1.84	1.56	7.71	30.	10.00
Control 2:					
Top	2.41	.97	1.80	31.2	5.83
Middle	2.58	2.19	9.44	40.	6.00
Bottom	2.75	2.17	4.98	30.8	13.33

The skim-milk controls of these experiments have not shown a constant predominance of bacteria on the surface. In fact, as a general rule, the greater number are to be found in the middle or the bottom of the bottle, indicating that the fat globules of the cream are the agents which dominate the peculiar distribution of bacteria in whole bottled milk. It may be mentioned in passing that the rate of multiplication of the bacteria in the lower portion of the milk is

considerably more rapid at low temperatures, at least, in the absence of a cream layer, possibly because of a freer access to the air oxygen.

INFLUENCE OF VARIOUS TEMPERATURES ON THE RELATIVE BACTERIAL CONTENT OF CREAM AND SKIM MILK.

In the comparative examination of a large number of bottles of milk, one finds that the ratio of the average count of the skim milk to that of the cream varies greatly. In certain samples the cream may contain over one hundred times as many bacteria as a like amount of skim milk, whereas in others the ratio of difference between the two portions may be less than two. Two factors are apparently of particular importance in determining the character of this relative distribution. First in import is the amount of primary contamination of the milk from the time it is drawn from the cow until it is bottled. If comparatively few bacteria gain access to the fluid the rising cream will incorporate a much higher percentage of them than when the primary contamination has amounted to several hundred thousand bacteria per c.c. A second factor which influences this relative bacterial content of cream and skim milk is the temperature at which the fluid is kept.

To determine the influence of this temperature factor a number of bottles of certified milk of high quality were placed at ice-box temperature (12° C.), at room temperature (18° to 23° C.), and at 30° C. At intervals samples from the cream and skim milk were withdrawn and plated. In Table 7 are detailed the average counts for five bottles at ice-box temperature. During the first 24 hours, the bacteria showed a tendency to decrease in number rather than multiply, but after 48 hours the rate of increase in the cream was somewhat greater than in the skim milk. Subsequently, to the time of souring, the rapidity of increase in the skim milk is only slightly in advance of that for the cream.

As the temperature at which the milk is kept is raised the relative rate of bacterial multiplication in the skim milk as compared with that in the cream becomes greater and greater. The average counts with five bottles of certified milk placed at room temperature are also given in Table 7. It will be observed that the relative rate of increase in the skim milk at 24 hours is considerably in advance of that in the ice-box series, altho this point is masked to some extent by sedimenta-

tion. As the souring-point is approached the rate of multiplication in the skim milk has so far outstripped that in the cream that the former now shows the greater degree of contamination. If one further raises the temperature of the environment to 31° C., the rate of increase in the skim milk may amount, within five hours, to many times that in the cream (Table 7).

TABLE 7.

INFLUENCE OF TEMPERATURE ON THE RELATIVE RATE OF INCREASE IN CREAM AND SKIM MILK.

BOTTLES AT ICE-BOX TEMPERATURE (12° C.).

Samples	Plated "At Once"	Plated after 24 Hrs.	Ratio to "At Once"	Plated after 48 Hrs.	Ratio to "At Once"	72 Hrs. Ratio to "At Once"	96 Hrs. Ratio to "At Once"	120 Hrs.* Ratio to "At Once"
1. Top cream.......	18,100	25,200	1.17	530,000	29.28	251	4,423	6,608
2. Middle cream	14,600	11,700	.80	86,000	6.02	265	3,327	7,285
3. Bottom cream....	10,400	7,400	.71	185,900	17.87	152	3,374	9,352
4. Top skim milk....	700	500	.71	5,900	8.42	178	1,974	11,794
5. Middle skim milk.	600	200	.33	2,000	3.33	333	976	8,366
6. Bottom skim milk.	300	600	2.00	8,200	27.33	566	27,706	75,040

BOTTLES KEPT AT ROOM TEMPERATURE (18°–23° C.).

Samples	Plated "At Once"	Plated after 5 Hours	Ratio to "At Once"	Plated after 24 Hours	Ratio to "At Once"	Plated after 48 Hours†	Ratio to "At Once"
1. Top cream..	28,600	35,600	1.26	14,744,000	526	113,900,000	4,069
2. Middle cream ...	21,200	27,900	1.31	14,202,000	676	184,000,000	8,761
3. Bottom cream	17,100	19,500	1.14	14,898,000	875	254,500,000	14,973
4. Top skim milk	900	1,200	1.33	368,700	409	219,600,000	244,000
5. Middle skim milk......	600	600	1.00	233,400	389	231,800,000	386,416
6. Bottom skim milk......	300	1,300	4.33	5,592,000	18,640	281,000,000	939,000

BOTTLE KEPT AT 31° C.

Samples	Plated "At Once"	Plated after 5 Hours	Ratio of Increase
1. Top cream.................	590,000	14,300,000	24.2
2. Bottom cream..............	370,000	10,140,000	27.4
3. Top skim milk.............	10,000	1,280,000	128
4. Middle skim milk...........	15,000	1,640,000	109
5. Bottom skim milk...........	1,000	1,625,000	1,625

* The cream and skim milk still sweet in all but one of the samples.

† Cream sour in all the bottles, skim milk in all but one.

DECREASE FOLLOWING SUDDEN CHANGE OF TEMPERATURE.

In investigating the effect of various temperatures on the relative increase of bacteria in the cream and the skim milk, it was observed that in some samples which were transferred from the ice-box to 30° C. and kept at that temperature for several hours there resulted a very

marked decrease in bacterial count in all parts of the bottle. Of eleven bottles so treated decided bacteriolysis occurred in three, a decrease in certain layers in three, and a rapid increase in bacterial count in five. An example of this last type is detailed in Table 7. In all samples in which the bacteria multiplied rapidly when transferred from 5° C. to 30° C. the initial count in the cream and skim milk was comparatively low. As has been observed, at 30° C. the bacteria in the skim milk multiplied far more rapidly than those in the cream and this increased rate of multiplication continued when placed at 12° C. for 18 hours. A very different result is shown in Table 8. In this instance the change from the cold to the warm environment resulted in the destruction of a large percentage of the bacteria. It seems probable that this was due to the liberation of mutually poisonous metabolic products by the various types of bacteria; in other words, it was an expression of antagonism. The milk was far too old (over 24 hours) to ascribe it to a germicidal action of the fluid itself. This antagonistic action occurred alone in samples with high counts in both the cream and the skim milk.

The bacteria in the skim milk recovered from this bacteriolysis more slowly than those in the cream. After 18 hours at 12° C. there was found to be a continued decrease in the count for the lean milk, whereas the bacteria at the top of the bottle had begun to multiply. Parallel litmus lactose agar plates exhibited a like primary decrease in the lactic acid bacilli. However, after exposure to the lower temperature they were found to have recovered more quickly than the bacteria thriving best in the more acid plain agar medium, and in fact from a comparison of the figures in the fourth column of Table 8 it is evident that they now constituted the dominant and practically exclusive type of bacteria in the milk. These dominant microorganisms were largely streptococci. If the bottle of milk is placed again at 30° C. no second bacteriolysis occurs.

An attempt was made to reproduce this antagonism experimentally by inoculating a sample of partially sterilized milk with a definite dosage of seven species of common milk bacteria, and submitting the mixture to these temperature changes. Altho there was some decrease in the count for the skim milk, the results were nothing like that detailed in Table 8. It is possible that this phenomenon under

natural conditions is dependent upon the development in milk of a peculiar and incompatible bacterial flora, which is not readily imitated experimentally.

TABLE 8.

MARKED BACTERIOLYSIS SOMETIMES OCCURRING IN BOTTLED MILK ON TRANSFERENCE FROM 5° C. to 30° C.

Samples	Plated "At Once"	Plated after 5 Hours at 30° C.	Ratio to "At Once"	Plated after 18 Hours at 12° C.	Ratio to "At Once"	Plated after 42 Hours at 12° C.	Ratio to "At Once"
Total count:							
1. Top cream	6,240,000	130,000	.0208	1,690,000	.27	15,000,000	2.40
2. Middle cream	2,285,000	45,000	.019	1,105,000	.48	28,000,000	12.25
3. Bottom cream	2.100,000	10,000	.0048	910,000	.44	21,320,000	10.25
4. Top skim milk	2,304,000	4,000	.0017	4,000	.0017	390,000	.16
5. Midde skim milk	2,145,000	24,000	.011	14,000	.0065	1,170,000	.54
6. Bottom skim milk	900,000	220,000	.244	19,000	.021	5,200,000	5.77
Lactic acid bacteria:							
1. As above	487,000	25,000	.051	1,170,000	2.40	....	.....
2. As above	266,000	20,000	.075	1,300,000	4.50	....	.....
3. As above	140,000	9,000	.065	620,000	4.28	....	.....
4. As above	70 000	......	.....	5,000	.07	390,000	5.57
5. As above	90,000	1,000	.011	7,000	.07	780,000	8.66
6. As above	26,000	......	.....	18,000	.69	1,160,000	44.5

The fact that the bacterial count in certain samples of rather grossly contaminated milk is materially reduced by an abrupt change in the temperature has apparently not been noted before. The point seems to be of some importance in the routine bacteriological examination of milk, for thereby a specimen of milk, which was primarily badly contaminated, might through the exercise of this antagonism simulate one which was obtained under proper cleanly conditions. During hot weather milk bottles in the interim between the filling at the dairy and the delivery to the customer are without doubt not infrequently subjected to as great changes in temperature as was the case in these experiments. Too few tests of this nature, however, were completed to give any idea of how often the bacterial conditions essential for this bacteriolysis obtain in milk.

SEDIMENTATION.

Contrary to what seems to be the general opinion, the sediment portion of the ordinary fresh bottle of milk embodies on the average fewer bacteria than any other portion of the fluid. This was found to be the case without exception in the certified brand of milk and frequently in the more grossly contaminated specimens. At the maximum the count was only slightly higher than the middle skim

milk and far below that of the cream. Sedimentation is evidently somewhat dependent on the rate at which the bacteria multiply in the skim milk as well as possibly on the temperature of the fluid. At low temperatures (Table 8) the increase in the sediment portion of the certified milk was not markedly greater than in the other parts of the skim milk until it had stood about 96 hours, whereas at room temperature the sediment at the end of 24 hours contained 25 times as many bacteria as the middle skim milk.

CONCLUSIONS.

1. The upper two ounces of the cream of fresh bottled milk of fair quality contain on the average 50 to 100 per cent more bacteria than an equal amount of the lower cream. In older and more grossly contaminated milk the lower cream may embody as many as or even more bacteria than the upper layers.

2. By removing these two top ounces from a milk bottle and using the remaining top milk (eight ounces) for infant feeding, as Hess has suggested, there generally results a reduction of from 30 to 50 per cent in the bacterial count.

3. The dominant controlling factor in the primary disposition of bacteria in a milk bottle is the upward "rafting" activity of the fat globules. A higher percentage of bacteria are brought to the surface layers in a milk rich in cream than one poor in that substance.

4. At ice-box temperature the rate of increase of bacteria in the cream and that in the skim milk are practically identical. As the temperature is elevated the rate of multiplication in the skim milk outstrips that of the cream until at 30° C. it may be many times as rapid.

5. In certain samples of rather highly contaminated milk the abrupt change in the temperature of the environment from 5° C. to 30° C. caused a striking bacteriolysis in both the cream and the skim milk. This was probably an expression of bacterial antagonism.

6. The sediment portion of the average bottle of fresh milk contains frequently fewer bacteria than any other region of the fluid. A marked excess of bacteria in the sediment indicates that the milk is old or that it has been kept in a warm place.